expression density in HEK cells high resolution TIRF microscopy was used to measure the intensity of individual fluorescent channels. Based on individual Kv2.1 intensity the average number of freely diffusing, non-clustered channels was ~200,000/cell whereas electrophysiological recordings of peak Kv currents corresponded to ~100,000 channels (n = 11), suggesting only 50% of the non-clustered channels are conducting K+. Endogenous Kv2.1 expression in cultured E18 hippocampal neurons at 20 days in vitro (DIV) was determined by immunocytochemistry, standardized to the GFP-Kv2.1 in HEK cells, and compared to total Kv current. Average immunofluorescence corresponded to ~60,000 channels (n = 11) in DIV 20 neurons while the ratio of clustered to non-clustered channels in DIV 20 neurons was 2:1 resulting in ~20,000 non-clustered channels. Steady-state Kv current magnitude in these neurons averaged 15 nA at +60mV (n = 4). Since 60% of this outward current is Kv2.1 mediated, there are 9,000 conducting Kv2.1 channels present. Thus, less than 50% of non-clustered Kv2.1 channels in cultured neurons conduct K+. These data also suggest that the endogenous Kv2.1 channels trapped within the cell surface clusters are held in a non-conducting state as observed in HEK cells.

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Effects of Electric Field on Channel Proteins Through Dipole Perturbation and Network of Signal Transmission

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Kv1.2 voltage-gated and MlotiK1 cyclic nucleotide-gated K+ channels belong to the family of tetrameric cation channels and share a similar protein fold in the transmembrane region. Kv1.2 channel is activated by the changes in the transmembrane potential, while MlotiK1 channel is activated upon the binding of cyclic nucleotides to its intracellular domain. We use a perturbation-based markovian transmission model [Lu and Liang,PLoS Comp. Biol. 2009] to study allosteric activation pathways in both channels. The initial perturbation on residues, e.g., ligand binding or conformational change, is converted to flow of probability, which allows studying of the time-course of signal transmission and propagation of probability flow through the protein molecule. As dipoles in channel proteins respond to the external electric field, change in energy and introduction of torque arise for individual residues. We postulate residues that experience large energy change and torque are those responding first to the membrane depolarization in ion channels. To identify regions of initial perturbation, we build structural models by embedding channel proteins in the POPC lipid bilayer, with surrounding slabs of water molecules on both sides of the membrane. Our calculations identified S1 helix of voltage sensing domain, linker, and filter regions in Kv1.2 channel, as well as helix S1 and linker in MlotiK1 channel as the regions of initial response, as they contain the majority of strongly polarizable dipoles. Our results show that dipole perturbation results in a strong signal transmittion to the charged arginine residues of S4 in Kv1.2, whereas no significant signal transmission is observed under the same perturbation for MlotiK1 channel. This suggests dipole perturbation is a mechanism how voltage gated channel proteins respond to external electric field. This mechanism, however, is not employed by ligand-gated channels.

Voltage-gated Ca Channels

3076-Pos Board B181

Cav3.1/α1G T-Type Ca^{2+} Channels are Involved in the Heart Rate Regulation

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T-type Ca^{2+} channels (TTCCs) are expressed in cardiac pacemaker cells and conduction system of mammals. However, the role of TTCCs in heart rate (HR) generation and regulation is not well understood. In the mouse, the major TTCC expressed in the heart is Cav3.1/α1G, and therefore we used Cav3.1/α1G transgenic (TG) and knockout (KO) mice respectively to define the role of TTCC in the heart rate generation and regulation. **Methods:** Telemetric (conscious) and surface (anesthetized) ECG were used to determine the effect of isoproterenol (ISO) on the HR *invivo*. To reduce the complication of *in vivo* HR regulation, Langendorff ECG was used to measure the response of the HR to ISO. Whole cell voltage clamp was used to measure the $I_{Ca\text{-}T}$ before and after ISO application on TG myocytes. **Results:** At baseline, telemetric ECG recording showed no significant difference in HR between the Cav3.1/α1G TG mice (536.3 ± 24.8bpm vs. FVB control: 550.6 ± 15.3bpm), Cav3.1/α1G KO mice (614.4 ± 39.9bpm vs. c57/bl6 control: 603.1 ± 64.5bpm) and control mice was detected. ISO increased the HR rate in conscious mice to the same extent in both TG (41.2 ± 6.9% vs. FVB control: 34.0 ± 3.6%) and KO (22.6 ± 8.8% vs. c57/bl6 control: 22.8 ± 8.5%) mice. However, when the central regulation is depressed (anesthetized) or removed (ex-vivo Langendorff perfusion), the percentage of HR increase after ISO application were significantly enhanced in the TG mice but reduced in KO mice. Cav3.1/α1G T-type Ca^{2+} currents ($I_{Ca\text{-}T}$) in sinoatrial nodal cells was significantly increased by 43 ± 16 % by ISO. **Conclusions:** Cav3.1/α1G TTCC might not play a major role in basal HR generation but it may play an important role in sympathetic/adrenergic regulation of HR, in which PKA could be an important mediator.

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Cav1.3 L-Type Calcium Channels-Mediated Ryanodine Receptor Dependent Calcium Release Controls Heart Rate

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Pacemaker activity of the sino-atrial node (SAN) controls heart rate. However, the mechanism underling SAN pacemaker activity is not completely understood and in particular, the respective physiological importance of ion channels and ryanodine receptor (RyR)-dependent Ca2+ release in pacemaking is hotly debated. We have investigated Ca2+ handling in SAN pacemaker cells of wild-type (WT) and mice lacking L-type Cav1.3 (Cav1.3-/-) channels. In isolated Cav1.3-/- SAN cells the frequency of Ca2+ transients was reduced by 45% compared to WT pacemaker cells. Loss of Cav1.3 channels also blunted by about 47% the positive chronotropic effect induced by 0.01 μM isoproterenol (ISO). Furthermore, in Cav1.3-/- pacemaker cells, local Ca2+ release (LCR) occurring during the diastolic phase was reduced by 71%. In SAN cells from mice in which L-type Cav1.2 channels have been rendered insensitive to dihydropyridines (Cav1.2DHP-/-), application of 0.3 μM isradipine decreased diastolic LCR by 78 %, thus showing that Cav1.3 channels are major regulators of RyR-dependent LCR during the diastolic phase. In individual cells of isolated intact SAN, pacemaking of Cav1.3-/- cells was characterized by reduced (37%) frequency of Ca2+ transients and an increase in Ca2+ waves. Normal pacemaking in Cav1.3-/- isolated SAN cells and intact tissue could be observed only after direct activation of RyR-dependent Ca2+ release by low doses of caffeine (200 μM). Experiments with high doses of caffeine (10 mM) in Cav1.3-/- cells, showed that the reduction in diastolic LCR and in the frequency Ca2+ transients could not be ascribed to a decrease in sarcoplasmic reticulum (SR) Ca2+ content. Our results show that in SAN pacemaker cell, LCR Ca2+ release is tightly controlled by Cav1.3 channels and that such a control is critical for promoting the formation of whole-cell Ca2+ transients.

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Evidence for a Role for the Cytoskeleton in Communication Between the L-Type Calcium Channel and the Mitochondria in Isolated Cardiac Myocytes

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Cytoskeletal proteins stabilize cell structure but also regulate subcellular distribution of mitochondria and cardiac L-type Ca^{2+} channel (LTCC) activity. We have previously demonstrated that mitochondrial function can be regulated by alterations in LTCC activity. This effect was attenuated when the cytoskeleton was disrupted with latrunculin A. To further explore this, we determined whether regulation of mitochondrial function by the LTCC is altered in a murine model of Duchenne Muscular Dystrophy(*mdx*). Mitochondrial membrane potential (Ψ_m) and metabolic activity was assessed after activation of the LTCC in cardiac myocytes isolated from C57BL/10ScSn-Dmdmdx/Arc (*mdx*) and C57BL/10ScSnArc (control) mice. Exposure of control myocytes to 10μM BayK(-) (LTCC agonist) caused a 11.4 ± 1.7% increase in Ψ_m assessed as alterations in JC-1 compared to myocytes exposed to inactive BayK(+) (n=8, $p<0.05$). The response was attenuated when myocytes were exposed to LTCC antagonist nisoldipine (n=7). However BayK(-) did not induce any significant alteration in JC-1 signal in myocytes from *mdx* mice (n=6). In control myocytes BayK(-) caused a 105.4 ± 7.4% increase in metabolic activity assessed using an MTT assay (n=8, $p<0.05$). The response was attenuated when myocytes were exposed to nisoldipine (n=8) or mitochondrial calcium uniporter inhibitor Ru360 (n=8) but unaltered when exposed to ryanodine receptor antagonist dantrolene (n=4). Exposure of *mdx* myocytes to BayK(-) did not induce any significant alteration in metabolic activity (n=8). These data confirm that alterations in LTCC activity can modulate mitochondrial function and that the cytoskeleton plays an important role in mediating this response. Since the LTCC is the initiator of contraction it has been proposed that a functional coupling between the LTCC and mitochondria may assist in meeting myocardial energy demand on a beat to beat basis.